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Fungal Taxa Target Different Carbon Sources in Forest Soil

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Abstract

Soil microbes are among the most abundant and diverse organisms on Earth. Although microbial decomposers, particularly fungi, are important mediators of global carbon and nutrient cycling, the functional roles of specific taxa within natural environments remain unclear. We used a nucleotide-analog technique in soils from the Harvard Forest to characterize the fungal taxa that responded to the addition of five different carbon substrates—glycine, sucrose, cellulose, lignin, and tannin-protein. We show that fungal community structure and richness shift in response to different carbon sources, and we demonstrate that particular fungal taxa target different organic compounds within soil microcosms. Specifically, we identified eleven taxa that exhibited changes in relative abundances across substrate treatments. These results imply that niche partitioning through specialized resource use may be an important mechanism by which soil microbial diversity is maintained in ecosystems. Consequently, high microbial diversity may be necessary to sustain ecosystem processes and stability under global change.

Key words: fungi; soil; microbial diversity; community structure; soil carbon; decomposition; resource partitioning; ecosystem function; nucleotide analog.

Introduction

An incredible diversity of microbes can be found in soil: one gram of soil can contain billions of microorganisms representing thousands of species (Torsvik and Øvreås 2002). How do so many species coexist in natural soil environments, where there is a limited amount of available space and resources? One mechanism responsible for the maintenance of diversity is the ability of coexisting species to vary in their patterns of resource utilization, resulting in a reduction in interspecific competition relative to intraspecific competition (Chesson 2000; Amarasekare 2003; Chase and Leibold 2003). As suggested by extensive theoretic work and some empirical studies, resource partitioning is likely to play a role in shaping plant and animal communities (Schoener 1974; Chesson 2000; McKane and others 2002; Chase and Leibold 2003). However, its importance in structuring microbial communities within a heterogeneous soil environment remains unclear (Zhou and others 2002; Amarasekare 2003).

Soil microbes can perform an immense range of metabolic functions. In fact, they can degrade almost every organic compound on the planet (Kjøller and Struwe 2002; Nannipieri and others

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2003). Fungi are especially successful in the decomposition of a wide variety of complex and recalcitrant plant-derived materials and are thus responsible for the breakdown of a large portion of the carbon stored within terrestrial ecosystems (Kjøller and Struwe 2002; Nannipieri and others 2003). For this reason, the relationship between ecosystem processes and microbial biodiversity has recently gained much interest (Loreau and others 2001; Hooper and others 2005; Wardle 2006). However, the degree to which functional diversity corresponds to taxonomic diversity for soil fungi, and whether this diversity is relevant at the ecosystem level remains unresolved (Zak and Visser 1996; Torsvik and Øvreås 2002; Wardle 2006). This uncertainty is likely due to the difficulty in characterizing the ecological functions of specific microbial taxa in situ (Torsvik and Øvreås 2002).

Much of what is known about the physiologic capabilities of soil microorganisms has come from a large body of culture-based studies using traditional microbiologic methods (Waksman and others 1928; Kjøller and Struwe 2002; Torsvik and Øvreås 2002; Deacon and others 2006). Mycologists have long investigated the range of carbon substrates ''consumed'' by various fungi, categorizing culturable fungi into broad functional groups (Waksman and Skinner 1926; Waksman and others 1928; Waksman and Nissen 1931; Garrett 1951). For example, Zygomycetes are recognized as sucrose and cellulose degraders, or ''sugar fungi,'' while some Basidiomycetes are identified as lignin-degrading ''wood rot fungi'' (Waksman and Skinner 1926; Waksman and others 1928; Waksman and Nissen 1931; Garrett 1951). These culture studies clearly indicate that the growth of certain fungi is limited by substrates that other fungal types proliferate on, suggesting that fungi specialize on different forms of carbon or plant material under simplified conditions.

Additionally, culture work combined with decomposition experiments have shown that the microbial community associated with decomposing plant material undergoes succession (Waksman and others 1928; Swift and others 1979; Frankland 1998; Kjøller and Struwe 2002; Robinson and others 2005), whereby microbial communities shift in response to progressive changes in resource availability. During forest litter decomposition, soluble or labile molecules, including inorganic nutrients, small amino acids, and simple carbohydrates, are targeted first, followed by the slow, progressive breakdown of more complex, recalcitrant plant materials such as lignin, tannin, and chitin (Swift and others 1979; Frankland 1998; Kjøller and Struwe 2002). As these different resources become available, corresponding changes occur in the composition of the dominant microbes associated with the decomposing litter: pathogenic microorganisms are first replaced by saprotrophic microfungi, followed by a dynamic and complex assemblage of Zygomycetes and Basidiomycetes with cellulose-, lignin-, and chitin-degrading capabilities (Frankland 1998; Kjøller and Struwe 2002). The changes in microbial community structure associated with this progression in chemical environment suggest that decomposer species exhibit varying resource utilization capabilities.

Although these studies may provide some evidence that resource partitioning is possible, culture-based studies are limited in defining microbial activities and their importance at the ecosystem level for several reasons. First, only a fraction of soil fungal species are culturable (Hawksworth 2001), meaning that the functional capacities of most fungal species remain unknown. Second, the culture environment represents growing conditions that rarely occur in the organism's natural environment. Naturally occurring soil microbes exist in complex assemblages of many coexisting species within heterogeneous, growth-limiting conditions (Amarasekare 2003; Zhou and others 2002). Such competitive conditions likely influence, or even constrain, the metabolic functions that microbial species perform under natural conditions (Amarasekare 2003). Thus, culture studies can only provide information on a small portion of the microbial diversity and activities occurring within natural soils (Hawksworth 2001). However, culture-independent, molecular-based techniques can be valuable tools for beginning to characterize the physiologies of unculturable organisms (Hawksworth 2001). Several studies have used molecular methods to describe the functional capabilities of soil microbes within an ecosystem context (Borneman 1999; Waldrop and others 2000; Yin and others 2000; Torsvik and Øvreås 2002; Waldrop and Firestone 2004), but directly linking the identities of diverse microbial species to specific resource use within natural soils remains challenging.

If microbial decomposers vary in their abilities to use different resources for growth in natural soil environments, then microbial diversity may be maintained in part by the ability of species to exploit different resource niches. We hypothesized that different taxa of fungal decomposers specialize in the breakdown of particular carbon substrates within soil. To test this hypothesis, we altered the availability of carbon resources within microcosms consisting of soil from the Harvard Forest and used a relatively novel molecular method to characterize

active fungal communities. Specifically, we quantified the relative abundance of fungal taxa that proliferated following the addition of a variety of carbon substrates to soil microcosms. We added glycine, sucrose, cellulose, lignin, or tannin-protein (in order of increasing resistance to decay) directly to sieved forest soils in conjunction with 3-bromodeoxyuridine (BrdU), a nucleotide analog. Active microbes absorb BrdU from the soil solution; if they multiply in response to substrate additions, they incorporate the BrdU into their newly synthesized DNA (Borneman 1999). After allowing soils to incubate for 48 h, we extracted BrdU-labeled DNA and sequenced the Internal Transcribed Spacer (ITS) regions of fungal ribosomal DNA (rDNA) to identify fungal taxa to near-species resolution. In total, we analyzed 1769 partial fungal ITS sequences. Fungal taxa that proliferated following a particular substrate addition were likely to be targeting the substrate as a resource for growth. Metabolic turnover of substrates by soil microbes was confirmed by incubating soils with ^{13}C-labeled substrates and measuring substrate-derived respiration. In this way, we were able to describe the resource utilization capabilities of different fungal taxa within a complex soil environment, in a culture-independent manner.

Materials and Methods

Sampling

We collected soils from the Harvard Forest Warming Experimental Site—Prospect Hill tract (Peterjohn and others 1994) in Massachusetts in September 2005. The forest at Prospect Hill is an even-aged mixed hardwood stand dominated by red maple (*Acer rubrum*), black oak (*Quercus velutina*), striped maple (*Acer Pensylvanicum*), and paper birch (*Betula papyrifera*). This site was chosen because it is a well-studied ecosystem that allows the opportunity to relate our results to those obtained in long-term manipulative studies at the site. Four soil cores were collected from the top 10 cm of the surface mineral layer of each of 12 plots. Six of these plots have been artificially warmed for 15 years using buried heating cables (plot #1, 6, 8, 12, 15, 16), whereas the other six plots were control plots consisting of buried cables, but have not been warmed (plot #3, 5, 9, 10, 13, 17). Since BrdU-labeling efficiency and subsequent PCR and cloning (see below) are challenging and particularly sensitive to DNA inhibitors from highly organic soils, we speculated that surface mineral soils would produce cleaner, more concentrated DNA yields and more reliable PCR products of consistent band intensity across replicates. For this reason, we chose to work with the surface mineral layer of the collected cores. The surface mineral soils from the Harvard Forest are still high in organic matter content, and thus are expected to contain metabolically active fungi.

Cores within each plot were homogenized and sieved through a 2.0-mm sieve. Composite samples were necessary to reduce the number of samples for molecular analysis while maintaining both an adequate spatial sampling, as well as a sufficient representation of the microbial diversity at the site. To create composite samples, we combined 10.0 g wet weight of the mineral layer of each of 3 randomly selected warmed plot samples, followed by homogenization, or we combined 10.0 g wet weight of the surface mineral layer of each of 3 randomly selected control plot samples, followed by homogenization. Altogether, we generated 4 replicate soil samples, 2 consisting of the soils from the warmed plots and 2 consisting of the soils from control plots. Replicate 1 consisted of an equal amount of the homogenized surface mineral layer soils from 3 randomly selected control plots (plot #5, 10, 17). Replicate 2 consisted of an equal amount of the homogenized surface mineral layer soils from 3 randomly selected warmed plots (plot #6, 8, 15). The same method was followed for replicates 3 and 4, except replicate 3 consisted of control plots #3, 9, 13; whereas replicate 4 consisted of warmed plots #1, 12, 16. Because this sampling and pooling scheme reduces plot treatment (warmed versus control) replicates, we could not test for warming effects in this study. However, this approach allowed us to test the carbon substrate use of a diverse range of fungi occurring within all plots at this site, regardless of plot treatment. Additionally, this sampling scheme means that any observed effects are likely conservative, owing to large variation among replicates.

Substrate Additions and Incubations for Analysis of Active Communities

Directly after combining soils as above, 2.0 g (wet weight) sub-samples were placed in sterile 50 ml tubes and 130 µl per g soil (wet weight) of one of six substrate mixtures was applied aseptically to the soil in each tube. The following six substrate mixtures were used: 7.69 mM 3-bromo-deoxyuridine (BrdU, Molecular Grade, Sigma-Aldrich) solution (control treatment); 7.69 mM BrdU containing 20 mg μl^{-1} of glycine (Molecular Grade, Fisher); 7.69 mM BrdU containing 20 mg μl^{-1} of sucrose

(Molecular Grade, Fisher); 7.69 mM BrdU containing 20 mg μl^{-1} of cellulose (microgranular cellulose powder, high purity, Sigma-Aldrich); 7.69 mM BrdU containing 20 mg μl^{-1} of lignin (alkali lignin, Sigma-Aldrich); and 7.69 mM BrdU containing 20 mg μl^{-1} of tannin-protein complex. Separate additions of sterile water only to a subset of soil samples were also performed as a method control (see Appendix A). Tannin-protein complexes were created from tannic acid (from plant material) and BSA (Molecular Grade, Sigma) by using the method described by Hagerman and Butler (1978). All water was filter- and UV-sterilized by a Simplicity 180 Water Purification System (Millipore). All chemicals were molecular-grade except lignin and tannin-protein, and sterile technique was used for soil handling and substrate applications. Tannic acid was isolated from natural plant materials, and the resulting tannin-protein complex was not sterilized before application to soils. Because the cellulose, lignin, and tannin-protein mixtures did not dissolve completely, we sonicated these mixtures for 10 min, and then before application to each soil sub-sample, we vortexed each mixture for 5 s. We also made sure to consistently pipette each mixture from the bottom of the tube using wide-orifice sterilized pipette tips to apply a consistent amount of substrate to each soil sub-sample. It was not necessary to completely dissolve these three mixtures because, as recalcitrant compounds, they would not be present in dissolved form in natural soils. After applying the mixtures using sterile technique, soils were gently mixed with sterile instruments, tubes were capped tightly, and soils were allowed to incubate for 48 h at room temperature in the dark. Mean daily soil temperatures at 10 cm depth were 21.3°C (control) and 25.6°C (warmed) in July, so an incubation temperature of approximately 22°C is a realistic approximation of the range of warm season temperatures experienced by these soil communities in nature.

DNA Extraction and Isolation of BrdU-Labeled DNA

Immediately following incubations, we extracted total DNA from 0.25 g (wet weight) of incubated soil using the PowerSoil DNA Isolation kit (MoBio Laboratories). To separate BrdU-labeled DNA from unlabeled DNA, we used the immunocapture method described by Yin and others (2000), with the following modification: all denaturation steps were performed at 100°C for 1 min, followed by flash freezing in an ethanol-dry ice bath. Our use of small soil samples could result in more variation among replicates, and we have used appropriate statistical tests to account for this variability throughout the study. Nonetheless, our use of small soil size contributes to our statistically conservative results and to our inability to exhaustively sample the entire community. However, this sampling strategy is sufficient for testing our hypothesis that different fungi utilize different carbon compounds in soil.

DNA Amplification and Clone Library Construction

BrdU-labeled DNA was amplified using the universal fungal primers ITS1-F (CTTGGTCATTTAGAGGAAGTAA) and ITS4 (TCCTCCGCTTATTGATATGC) (White and others 1990). Reactions were performed in duplicate on an iCycler thermocycler (BioRad, see Appendix A). To reduce PCR primer bias, we used the same PCR cycling conditions for all samples, included BSA in the reactions, reduced the number of cycles to the lowest possible, and performed PCR reactions in duplicate. Products from duplicate reactions were pooled in equal volumes and verified by agarose gel electrophoresis to confirm that PCR products from all samples were of equal intensity. Target amplicons (see Appendix A) were then purified from the gels by using a QiaQuick Gel Extraction kit (Qiagen) and cloned using a TOPO-TA Cloning kit for Sequencing (Invitrogen). We created clone libraries consisting of one 96-well plate per substrate-incubated soil sample, for a total of 2304 clones. One-directional high-throughput sequencing was performed by Agencourt Bioscience Corp. on ABI PRISM 3730xl sequencers.

Relative Fungal Abundance

To estimate fungal abundance, we performed quantitative PCR (qPCR) on total DNA by using the fungal-specific (partial 18S rRNA gene) primers nu-SSU-0817-5′ (TTAGCATGGAATAATRRAATAGGA) and nu-SSU-1196-3′ (TCTGGACCTGGTGAGTTTCC) (Borneman and Hartin 2000). Triplicate reactions contained 400 nM of each primer, 0.5 µl SYBRGreen (BioRad) per µl reaction mixture, and 0.1 µl DNA template per µl reaction mixture. Reactions were carried out on a MYiQ single color real-time PCR detection system (BioRad) with the following cycling conditions: initial denaturation at 95°C for 15 min, followed by 37 cycles of 15 s denaturation at 94°C, 30 s annealing at 56°C, 30 s elongation at 72°C. Assays were carried out in 96-

well plates. Each sample was amplified in triplicate, and bulk DNA extracted from the study soils was used to construct standard curves. A melting curve analysis was performed after each analysis to confirm the specificity of the qPCR. To generate melting curves the following heating conditions were applied: 55°C starting temperature increasing by 0.10°C increments for 80 cycles, followed by a 10 min final elongation step at 72°C and holding temperature at 4°C. qPCR results are expressed as relative abundance, scaled to the quantity of fungal DNA observed in the control microcosms. Analysis was performed with MYiQ software (BioRad). We used a Kruskal–Wallis test ($\alpha = 0.05$, $n = 4$) to determine significance among treatments.

Substrate-Induced Respiration

To confirm that microbes were metabolizing the added substrates, we performed a separate incubation experiment using ^{13}C-labeled substrates. Soils were adjusted to 50% water-holding capacity and then 2 g dry weight equivalent was placed in 50-ml centrifuge tubes, capped by lids modified to accommodate an o-ring and septum for headspace gas sampling. Substrates (^{13}C-labeled) were added in solution (glycine and sucrose) or as a fine powder (lignin and cellulose); ^{13}C-enrichments, relative to the soil-derived CO_2 with a C_3-plant value (−28.4‰) were −11.6, 956, 246, and 268‰ for sucrose, glycine, lignin and cellulose. For lignin and cellulose additions, deionized water was added to bring the soils to 65% water-holding capacity, as in the other treatments. Substrate addition amounts were predetermined using a dose-response procedure to ensure that they did not become limiting (to mineralization) during the assay period. Cellulose and lignin were purified from ^{13}C-labeled oak foliar material by exposure of the senesced material to diglyme-HCl and H_2SO_4 (72%), respectively; sucrose and glycine were purchased (Sigma). Soils receiving water only were included to determine the δ^{13}C-values of soil-derived CO_2. All assays were run in duplicate (that is, two analytical repeats; given the reduced variation between analytical repeats using the ^{13}C-label approach, duplicate as opposed to triplicate assays were sufficient).

Immediately following substrate/water addition, centrifuge tubes were capped and flushed with CO_2-free air to bring headspace CO_2 concentrations to less than 1 μl l^{-1}. Soils were then incubated at 20°C for 24 h, when the headspace was sampled using a gas-tight syringe (SGE) and then transferred to an Exetainer (Labco) for isotope analysis, or directly injected into a sample-loop connected to a continuous stream of CO_2-free air passing through an LI-7000 infrared gas analyzer (LiCor) to determine CO_2 concentration. We did not incubate soils for 48 h, as in the DNA analyses, because after 24 h potential substrate mineralization rates declined and so 24 h incubations give information on maximum, potential rates. Headspace samples were analyzed for ^{13}C/^{12}C ratios by continuous-flow, isotope ratio mass spectrometry (Thermo, San Jose, USA) at the Analytical Chemistry Laboratory of the Odum School of Ecology, Univ. of Georgia. Analytical precision was ± 0.1 $\delta^{13}C‰$. Working gas standards and solid reference materials were calibrated to PDB using NIST-SRM 1577b Bovine Liver as a reference standard.

To calculate the proportion of respired CO_2 derived from the substrates the following mixing equation was used:

$$C_{\text{substrate-derived}} = C_{\text{pool}} * (\delta^{13}C_{\text{pool}} - \delta^{13}C_{\text{soil}})/(\delta^{13}C_{\text{substrate}} - \delta^{13}C_{\text{soil}})$$

where C_{pool} and $\delta^{13}C_{\text{pool}}$ are the measured size and δ^{13}C values of the headspaces from substrate-added assays, $\delta^{13}C_{\text{soil}}$ is the δ^{13}C value of the CO_2-derived in the water only addition assays, and $\delta^{13}C_{\text{substrate}}$ is the δ^{13}C value of the added substrates.

We tested for significant treatment effects using Kruskal–Wallis tests followed by post-hoc mean separations with Kolmogorov–Smirnov tests ($\alpha = 0.05$, $n = 11$ for cellulose and lignin, $n = 12$ for glycine and sucrose).

Sequence Analysis

Sequences were edited and trimmed using the program BioEdit (Hall 1999), and aligned by hand in the program ClustalW (Chenna and others 2003). We trimmed sequences to an approximately 250 bp region including about 30 bp of the 18S gene, the entire ITS1 region, and approximately 30 bp of the 5.8S gene (see Appendix A). The conserved regions at either end of the resulting sequence fragment (either 18S or 5.8S) served to facilitate alignment. After elimination of unreliable sequence reads and short or ambiguously aligned sequences, 1769 sequences remained for alignment and analysis. The alignment was used to generate distance matrices in the Phylip program DNADIST (Felsenstein 2005) with an F84 evolutionary model and a transition:transversion ratio of 1.0. For taxonomic identification, we used BLAST searches to match the DNA sequences with known organisms in Genbank (see Appendix A). Sequences used in the alignment have been deposited in Genbank under the accession numbers EU805932-EU807700.

OTU Analysis and Statistics

Matrices of genetic distance from DNADIST were input to the software program DOTUR (Schloss and Handelsman 2005) and used to generate operational taxonomic units (OTUs) containing sequences with 97% or greater similarity based on a furthest-neighbor algorithm. All subsequent community analyses were performed as previously described by Allison and others (2007). Briefly, for each substrate treatment, we used DOTUR to calculate rarefaction curves of OTU richness, Chao1 estimates of richness (Chao 1984), and Shannon Indexes of diversity (Magurran 1988) as a function of sequences sampled. We tested for significant treatment effects on these parameters by checking for non-overlapping 95% confidence intervals (Table 1). We also used DOTUR to generate rank abundance curves for each treatment and to determine the frequency of rare taxa (defined as 1 or 2 sequences per treatment) under each substrate.

We examined active fungal community structure within each substrate treatment using multivariate tests on relative abundances of OTUs from DOTUR. Following Allison and others (2007), we created non-metric multidimensional scaling (NMS) ordinations and performed statistical tests of treatment effects by multi-response permutational procedures (MRPP) (McCune and Grace 2002) using the statistical package R (R Development Core Team 2006). We tested for treatment effects on the relative frequencies of individual OTUs using non-parametric Kruskal–Wallis tests. Because this test is conservative and the MRPP analysis was significant, we did not adjust *P*-values for multiple comparisons. For all statistical tests, differences were considered significant at $P < 0.05$, and marginally significant at $P < 0.10$.

Results and Discussion

We found that the structure of active fungal communities varied significantly among substrates. According to MRPP analysis, the active fungal community under the glycine treatment was significantly different from those under cellulose, tannin-protein, and control conditions ($P = 0.027$, 0.030, 0.028, respectively), whereas the active communities under sucrose and cellulose were marginally different from each other ($P = 0.058$) and the control ($P = 0.059$, 0.054, respectively). An ordination plot produced by NMS analysis (Figure 1) largely paralleled the MRPP analysis, as shown by the distinct groupings of the active

Table 1. Richness, Diversity, Total Abundance, and Substrate-Derived Soil Respiration

	Control	Cellulose	Glycine	Lignin	Sucrose	Tannin-protein
Total sequences analyzed	275	307	296	283	306	302
Total OTUs[1]	33	46	33	44	34	31
Chao1 estimate of OTU richness[1] [95% Confidence interval]	39 [34, 55][a]	60 [47, 107][a]	50 [37, 97][a]	58 [48, 91][a]	35 [32, 50][a]	54 [36, 128][a]
Shannon index of diversity[1] [95% Confidence interval]	2.42 [2.26, 2.58][a]	3.23 [3.12, 3.34][c]	2.34 [2.18, 2.50][a]	2.87 [2.72, 3.02][a]	2.63 [2.49, 2.77][ab]	2.64 [2.51, 2.77][ab]
Relative fungal abundance[2] (qPCR) (± s.e.m., $n = 4$)	1.00 ± 0.28	1.70 ± 0.60	2.45 ± 1.03	1.59 ± 0.63	1.32 ± 0.21	1.47 ± 0.34
Substrate-derived CO_2[3] (μg CO_2-C g $soil^{-1}$ d^{-1} ± s.e.m., $n = 11$, 12[4])	N/A	22.48 ± 2.96[a]	11.42 ± 1.14[b]	4.38 ± 0.67[c]	18.45 ± 2.52[a]	ND

[1]*Total number of OTUs, richness, and diversity were based on OTU designations of active fungal ITS sequences at the 97% or greater sequence similarity level. Chao1 estimates and Shannon indexes with different letters have non-overlapping 95% confidence intervals.*
[2]*Values scaled to DNA quantity in control microcosms. No significant differences.*
[3]*Substrate-derived CO_2 was obtained after incubating soils with ^{13}C-labeled substrates for 24 h and analyzing respired CO_2 for isotope content. Substrate-derived CO_2 values with different letters are significantly different (Kolmogorov–Smirnov pairwise comparisons).*
[4]*$n = 11$ for cellulose, lignin; $n = 12$ for glycine, sucrose.*

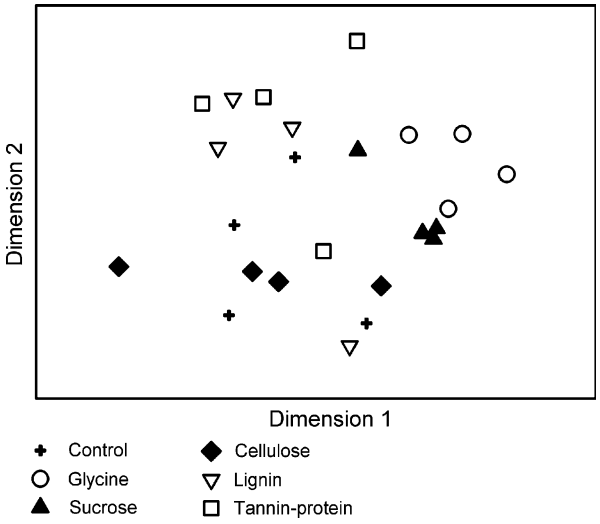

Figure 1. Non-metric multidimensional scaling (NMS) plot at 97% or greater sequence similarity for active fungal communities from soils receiving organic substrate additions.

communities associated with glycine and sucrose. The communities under cellulose also clustered together, but only along Dimension 2. These results indicate that the overall community structure of active fungi was altered by the addition of glycine, sucrose, and cellulose and imply that some fungi respond to shifts in carbon resource availability.

Within these active fungal communities, we identified 11 operational taxonomic units (OTUs, ≥97% sequence similarity level) that exhibited marginal changes in relative abundance under different substrates (Figure 2). The majority of these taxa responded positively to the addition of glycine (OTUs 5, 8, 17, 120) and sucrose (OTUs 5, 13, 18, 25, 52), which are labile soil compounds that are relatively abundant and easily metabolized by microbes. However, several taxa proliferated under the more complex and recalcitrant substrates: OTU 25 responded positively to cellulose, whereas OTUs 18 and 47 proliferated under lignin. Although some taxa had the capacity to respond to a diverse set of resources (OTUs 5, 8, and 18), others appeared to target a very specific carbon source, such as glycine (OTUs 17, 42, 120) or lignin (OTU 47). It is important to note that, with the exception of one taxon (OTU 5), these single-species responses are only marginally significant. Although our statistically conservative sampling strategy likely contributed to this, we were still able to detect significant whole-community changes in response to the added substrates. These results demonstrate that fungal taxa exhibit different

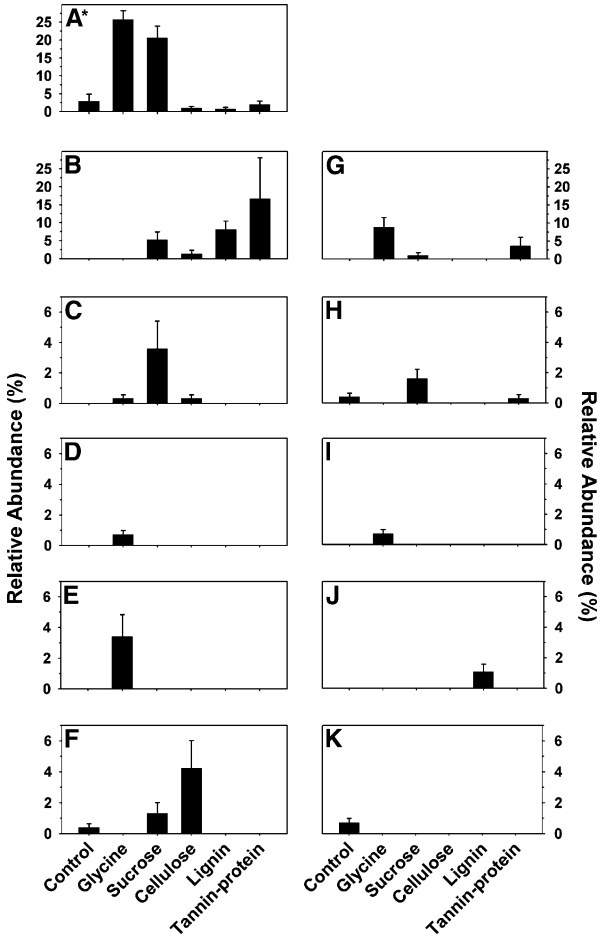

Figure 2. Relative abundances (% of sequences within a treatment) of active fungal OTUs showing at least a marginally significant change in relative abundance ($P < 0.10$, except * = $P < 0.05$, Kruskal–Wallis tests) among substrate additions. In total, only 11 OTUs exhibited significant or marginally significant changes. (**A**) Zygomycete (Mortierellaceae), OTU 5. (**B**) Zygomycete (*Mortierella*), OTU 18. (**C**) Zygomycete (Mortierellaceae), OTU 13. (**D**) Zygomycete (Mortierellales), OTU 17. (**E**) Zygomycete (*Umbelopsis*), OTU 42. (**F**) Unknown fungus, OTU 25. (**G**) Unknown fungus, OTU 8. (**H**) Unknown fungus, OTU 52. (**I**) Unknown fungus, OTU 120. (**J**) Basidiomycete (Agaricales), OTU 47. (**K**) Basidiomycete (*Lactarius*), OTU 77. OTUs were determined by analysis of sequences at the 97% or greater similarity level. Bars represent s.e.m., $n = 4$.

resource utilization capabilities and that some are able to specialize in the breakdown of particular compounds in soil microcosms. Together, this suggests that co-occurring fungal decomposers occupy different carbon resource niches within complex soil communities.

The taxonomic identities of the eleven OTUs, as characterized by comparison to known sequences in Genbank, were consistent with known physiol-

ogies of closely related taxa as determined from culture studies. For example, four of the eleven OTUs belonged to the phylum Zygomycota and the order Mortierellales (Figure 2A–D), which comprise one of the most functionally diverse groups of saprotrophic microfungi found in soils (Kjøller and Struwe 2002). Although many Zygomycota are considered primary colonizers in the decomposition of litter and relatively minor players in the breakdown of recalcitrant plant material, cellulose- and lignin-degrading capabilities have been observed in some culturable members of the order Mortierellales (Kjøller and Struwe 2002). This is consistent with our finding that different taxa within the order Mortierellales exhibit a wide range of functional capabilities, responding positively to both labile and recalcitrant substrate additions. Additionally, OTU 47 (Figure 2J), which responded to lignin, belonged to the phylum Basidiomycota and the order Agaricales. Basidiomycetes, including members of Agaricales, are widely recognized wood decomposers and producers of the enzymes responsible for lignin degradation (Waksman and Nissen 1931; Garrett 1951; Kirk and Farrell 1987; Kjøller and Struwe 2002; Deacon and others 2006).

To confirm that the community composition shifts and specific taxon responses could be attributed to microbial utilization of the added substrates, we measured respiration of ^{13}C-labeled glycine, sucrose, cellulose, and lignin from the soils (Table 1). After allowing soil samples to incubate with the labeled substrates for 24 h, isotope analysis of respired CO_2 revealed that substrate-derived respiration was detectable for all substrates (that is, significantly greater than zero, *t*-tests, $P < 0.001$ in each case). These data confirm that soil microbes were acquiring carbon from the added substrates, and then releasing it as respired CO_2. As expected, substrate-derived respiration was greatest upon the addition of the carbohydrates—cellulose and sucrose—which consist of large amounts of metabolically available carbon (Kruskal–Wallis test, $P < 0.001$).

Although some taxa proliferated as a result of substrate additions, others declined. For example, all substrates reduced the relative abundance of OTU 77 as compared to the control (Figure 2K). We identified this OTU as the genus *Lactarius*, a well-known Basidiomycete. We also found a decrease in the fraction of rare OTUs (abundance of 1 or 2 sequences within a treatment at ≥97% similarity) within the active fungal communities upon the addition of all substrates: control (61%) > lignin (57%) > glycine (55%) > cellulose (46%) > sucrose (44%) > tannin-protein (35%). This pattern may be a result of competitive exclusion, whereby fungi that specialize on the added substrates out-compete other taxa.

The richness and diversity of active fungal communities also varied among substrate treatments. Specifically, cellulose and lignin additions elicited the greatest richness of active fungal taxa, a pattern reflected by both Chao1 richness estimates and observed numbers of OTUs (Table 1, Supplementary Figure B1). Likewise, Shannon diversity indices revealed a significant increase in the diversity of active fungi under cellulose and lignin additions (Table 1). Moreover, the decreasing slopes of rarefaction curves (Supplementary Figure B1) indicate that the diversity of active fungi within these communities was sampled sufficiently. These results imply that a more diverse assemblage of fungi may be responsible for the breakdown of these complex soil molecules compared to more labile compounds. This finding also agrees with the prediction that the decomposition of polymeric, recalcitrant compounds such as cellulose and lignin requires a diverse suite of enzymes (Kirk and Farrell 1987; Lynd and others 2002), each of which may be produced by different species (Schimel and Gulledge 1998) and may act at different sites within the targeted molecule. A relatively high diversity of fungi may be supported if different taxa specialize in the degradation of different components of these compounds.

Estimates of relative fungal abundance, as measured by quantitative polymerase chain reaction (qPCR) on total fungal DNA (active and inactive fungi), revealed that substrate additions did not significantly alter the abundance of fungi relative to control (Table 1). Nevertheless, relative abundance tended to increase with the addition of all carbon substrates, particularly glycine. The trend toward greater fungal abundance under this substrate is likely explained by the notable prevalence of OTU 5, which represented over 25% of the active community under glycine (Figure 2A).

Our results demonstrate the utility of a novel nucleotide-analog technique to pinpoint active microbes within soil microcosms. However, our approach is not free of caveats. First, our sampling design and use of small soil sizes impart high variability among replicates within all treatments, making it difficult to detect significant responses. Second, disruption to the soil microbes is a concern for any laboratory-based study on soil communities. Coring and sieving likely reduced the activity of fungi with extensive hyphal networks, and our results may be biased toward opportunistic, fast-growing fungi, or fungi with the capacity to

germinate quickly from spores. Despite this, we successfully detected actively growing, and ecologically relevant decomposer fungi using this approach. It is also important to recognize that these microcosm soil communities do not allow an exhaustive sampling of the entire fungal community, and our diversity results are undoubtedly underestimates of the actual fungal diversity within soils from our sites. However, discerning the resource capabilities of all fungi within a soil community is an insurmountable task with currently available methods. Because the microcosm microbial communities were not artificially assembled as in culture-based approaches, they are representative of partially intact, diverse native soil communities. Thus, our approach is a useful culture-independent method for detecting the potential functions of microbes under the competitive environment of a complex soil microbial assemblage.

Although we examined fungi that could respond to substrate additions only within 48 h, we expect this timeframe to correspond with the frequency of natural changes in resource availability that would occur regularly in the Harvard Forest. For example, frequent litter fall or precipitation events can quickly alter the soil resource environment at both the microsite and ecosystem scales. Recurrent variations in microbial activities are therefore expected to occur naturally in these soils in response to such events. Indeed, rapid shifts in microbial colonization and activities have been observed previously for soil bacteria (Marschner and Rumberger 2004). Moreover, 48 h was ample time for microbes to metabolize the added substrates, as indicated by the significant release of ^{13}C-labeled CO_2 upon the addition of labeled substrates after only 24 h (Table 1). We experimentally shifted resource availability in these soils and used molecular methods to pinpoint active microbes. In this way, we were able to identify fungal taxa capable of exploiting newly available resource niches within a relatively quick but ecologically relevant temporal context.

Decomposer microbes are vastly diverse both taxonomically and functionally, yet linking the contributions of specific soil microorganisms to ecosystem-level processes remains one of the biggest challenges for both ecosystem and microbial ecologists (Zak and Visser 1996; Torsvik and Øvreås 2002; Wardle 2006). Because soil microbes act as a control over the breakdown of a major terrestrial carbon stock, understanding the physiology and ecology of these organisms in their natural state is critical for global change predictions (Zogg and others 1997; Wolters and others 2000). Although this microcosm study was performed under controlled laboratory conditions on soils removed from their natural state, our study highlights the utility of a novel nucleotide-analog approach for linking microbial identity to potential ecological function within complex soil communities. Furthermore, the identification of the particular roles of decomposer fungi within the Harvard Forest is a valuable contribution to our growing understanding of the controls over biogeochemical cycling within forest ecosystems. Future research should examine how fungal taxa change in abundance and activity under soil warming at this site.

Overall, our results demonstrate that fungal communities change in response to various carbon sources and that fungal decomposers may specialize in the breakdown of particular organic compounds within soil microcosms, providing evidence in support of our hypothesis. Because fungal taxa within a complex community did not respond equally to the applied resources in our microcosm study, we further suggest that resource partitioning may be an important mechanism for structuring soil microbial communities in natural environments. However, there are many factors that may promote the coexistence of diverse microbes in soil, such as spatial and temporal variation in resource availability across a heterogeneous landscape, or trade-offs that involve interspecific compromises between competitive ability and susceptibility to grazers, viruses, or environmental changes (Bohannan and others 2002; Zhou and others 2002; Amarasekare 2003; Wardle 2006). Recent culture and field experiments have reinforced the importance of environmental and competitive pressures as controls over fungal diversity (Toljander and others 2006; Waldrop and others 2006; Wardle 2006). Our results provide evidence for resource partitioning among soil fungi within a laboratory setting and emphasize the need for future studies that examine the relative importance of this mechanism for facilitating species coexistence within natural soils.

If decomposer species partition their resource use within natural ecosystems, and do not equally utilize resources, then relatively high taxonomic diversity among decomposer microbes may be important for maintaining carbon and nitrogen turnover within soils. Furthermore, maintenance of these biogeochemical processes may be necessary for ecosystem functioning and stability (Wolters and others 2000; Loreau and others 2001; Hooper and others 2005). Because global change can alter microbial biomass, community composition, and activity (Zogg and others 1997; Wolters

and others 2000; Treseder 2004), the identification of microbial species that are critical for soil processes is essential for the preservation of entire ecosystems. This has important implications for conservation efforts, as the loss of certain fungal taxa may result in shifts in carbon and nutrient cycling under global change (Zak and Visser 1996; Chapin and others 1997; Schimel and Gulledge 1998; Loreau and others 2001; Hooper and others 2005).

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